AD_____

Award Number: W81XWH-12-2-0122

TITLE: Pediatric Susceptibility to Nerve Agent-Induced Seizures and Effectiveness of Anticonvulsant Treatments

PRINCIPAL INVESTIGATOR: Francis Edward Dudek

CONTRACTING ORGANIZATION: University of Utah

Salt Lake City, UT 84112-9023

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 Oct-2013	Annual	26 Sep 2012 – 25 Sep 2013

4. TITLE AND SUBTITLE

Pediatric Susceptibility to Nerve Agent-Induced Seizures and Effectiveness of Anticonvulsant Treatments

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-2-0122

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Erika A. Scholl, F. Edward Dudek

E-Mail: Erika.scholl@hsc.utah.edu, ed.dudek@hsc.utah.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Utah
201 S Presidents Circle Rm 408
Salt Lake City, UT 84112-9023

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Organophosphate (OP) poisoning can result in status epilepticus (SE), a medical emergency which can become pharmacoresistant if treatment is delayed. Little to no data exists on pediatric models of OP-induced SE, even though the immature brain is likely to respond differently to OPs, and the optimal therapies are also likely to differ from adults. Our aim is to identify novel drugs that block pharmacoresistant OP-induced SE in children. In the past year, we have provided USAMRICD investigators with the miniature telemetry devices and recording apparatus for recording from P7 and P14 rats. We have developed rat models for DPF exposure for ages P7, P14, P21, and P28. We have found that P7 and P14 rat pups have brief (minutes) seizure behavior in response to DFP, while P21 and P28 animals develop SE which is robust and lasts for several hours. We see extensive neuronal injury in P28 rats using Fluoro-Jade B as a marker. We have developed an electrographic profile for the EEG activity of DFP-treated P21 and P28 animals, and are working on profiles for P7 and P14 DFP-treated rat pups. We have begun characterizing the effects of midazolam on DFP-induced SE in P21 and P28 rat pups, and we find that midazolam ameliorates the SE triggered by the organophosphate.

15. SUBJECT TERMS
Status Epilepticus, seizure, organophosphate, DFP, pediatric, neuronal injury, anticonvulsant, midazolam

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Organophosphate (OP) poisoning can result in status epilepticus (SE), a medical emergency which can become pharmacoresistant if treatment is delayed. Little to no data exists on pediatric models of OP-induced SE, even though the immature brain is likely to respond differently to OPs, and the optimal therapies are also likely to differ from adults. Our aim is to identify novel drugs that block pharmacoresistant OP-induced SE in children.

EEG monitoring will involve a novel miniature telemetry device, which will allow freely-moving behavior, ultimately while pups are cohoused with the dam. Neuropathological data will be analyzed with FluoroJade B, a marker for dying neurons. Pharmacological agents to be tested are diazepam, midazolam, phenobarbital, fos-phenytoin, and propofol.

Statement of Work

1. Provide USAMRICD researchers telemetry devices and recording apparatus to record EEG from pediatric rats for the nerve agent experiments at USAMRICD
2. Develop animal models of nerve agent exposure in immature (post-natal days 7, 14, 21, and 28) rats using Li-pilocarpine and the organophosphate DFP seizure models to record electrographic status epilepticus. Characterize the electrographic characteristics of these seizures and determine the neuropathological effects of these seizures in various brain areas.
3. Once these models have been developed and characterized, they will be used to cross-validate the anticonvulsant and neuroprotective properties of up to five FDA-approved or investigational drugs in these models. The drugs initially proposed for testing are diazepam, midazolam, phenobarbital, fosphenytoin, and propofol.

Body

Investigators at the USAMRICD were provided with miniature telemetry devices and recording apparatus for examining EEG in neonatal and juvenile rats.

Initial studies were performed to probe the toxic profile of the organophosphate, diisopropylfluorophosphate (DFP) in postnatal day (P) 7, 14, 21, and 28 day rat pups not implanted with the miniature telemetry device.

Dose (mg/kg)	Age	n	% behavioral seizures	% mortality (24 hrs)
1	P7	4	100	0
	P14	4	100	0
	P21	4	0	0
	P28	3	0	0
2	P7	4	100	0
	P14	4	100	0
	P21	4	100	0
	P28	4	100	0
3	P21	4	100	0
	P28	4	100	50
4	P21	4	100	0
	P28	3	100	0

Table 1. Summary of DFP dose-ranging studies in unimplanted pups.

Animals of all ages showed the following toxic signs in their behavioral response to DFP: head-bobbing, whole-body tremor, myoclonic jerks, lip smacking, gagging, gasping, urination, defecation, excessive salivation, and Straub tail. Piloerection, hyperlacrimation and exopthalmos were observed in P21 and P28. Vocalizations were audible in P7 and P14. Due to the immaturity of P7 limb development, whole body arching was observed only at this age. Similarly, swimming motions of the limbs were seen only in P7 pups. In general, the younger the animal, the more quickly the toxic signs disappeared. Also, mortality was most often observed within the first 10-15 minutes following the DFP, most likely due to the central suppression of respiration.

We found that the dose used to elicit apparent behavioral seizures in unimplanted P28 animals, with minimal mortality (3-4 mg/kg), was below the threshold for eliciting electrographic SE for most implanted rats. At 4 mg/kg, we observed epileptiform activity in some animals, which in one case progressed to SE (Table 1). Figure 1 illustrates an episode of electrographic SE in a P28 rat pup. Electrographic activity and subsequent behavioral SE was more consistently observed with 5.5-6.5 mg/kg DFP (Table 2), although more mortality occurred. These data suggest that (1) lower doses of DFP, which led to little or no mortality, did not consistently cause electrographic seizures, and (2) higher doses of DFP more frequently caused both electrographic seizures and mortality. Also, intracerebral recordings of local field potentials are needed to confirm that events that appear to be behavioral seizures are actually electrographic seizures.

DFP Dose (mg/kg)	Behavioral Seizures	Electrographic Seizures	SE	Mortality
3.0 (n=3)	100%	0%	0%	0%
4.0 (n=7)	100%	57%	14%	0%
5.5 (n=4)	100%	100%	75%	50%
6.0 (n=8)	100%	100%	63%	25%
6.5 (n=12)	100%	58%	25%	42%

Table 2. Summary of DFP dose-ranging studies in telemetry-implanted P28 rat pups.

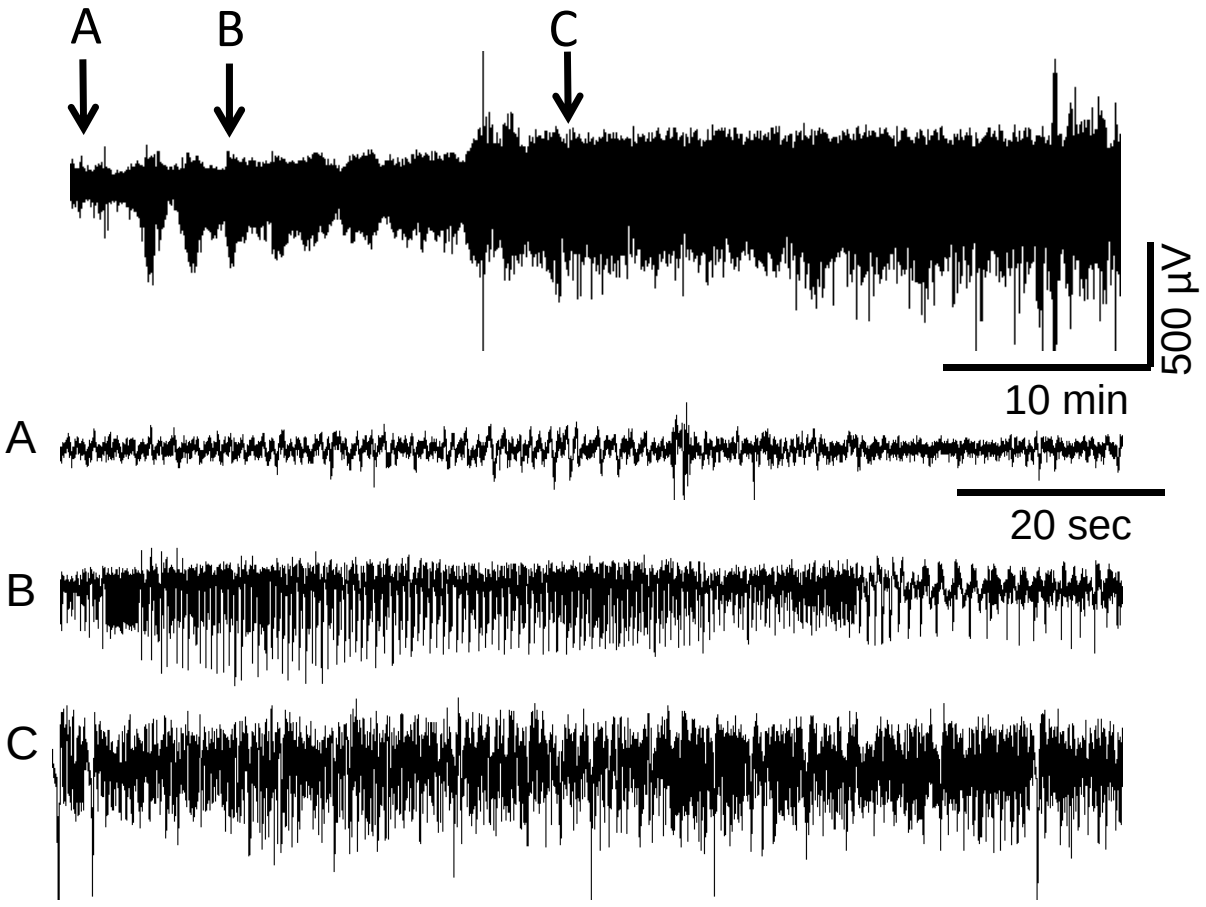

Figure 1. DFP-induced status epilepticus in a P28 rat. Animals were treated with 0.026 mg/kg pyridostigmine bromide (i.p.) 30 min prior to dosing with 5.5 mg/kg DFP (s.c.). At 1 min following DFP administration, animals were given 0.1 mg/kg atropine sulfate admixed with 25 mg/kg 2-PAM (i.p.).

One problem encountered was that the DFP dose-ranging studies from P28 animals suggest that mortality could be as high as 25-50% under conditions where electrographic seizure activity can be studied with pharmacological agents. Furthermore, behavioral activity, which appeared to be seizures, has been observed without evidence for electrographic seizures.

After examining P28 rat pups, we turned to P7, P14, and P21 rats and were able to elicit and record electrographic seizures (Fig. 2, Table 3) from them. In P21 animals, a dose of 5-5.5 mg/kg DFP was sufficient to evoke SE, which was qualitatively similar to the SE observed in P28 pups (Fig. 1). SE

was robust and lasted several hours. Also, as in P28 animals, P21 pups sometimes displayed uncoupled clinical and EEG behavior. Behavioral and electrographic seizures were also observed in P7 and P14 pups, at a dose of 4-4.5 mg/kg DFP (Table 3). Whereas the electrographic activity in P21 and P28 animals began after a delay of 5 or more minutes, in P14 animals the delay was shorter, and in P7 animals the electrographic seizure activity began almost immediately (Fig 2). Whereas the SE was robust and apparent for several hours in P21 and P28 animals, in P14 pups the SE spontaneously terminated after about 1 hour and in P7 animals seizure activity was gone after 15-30 minutes (Fig 2). These data suggest that P7 animals may not have the molecular circuitry that allows for self-sustaining SE that is observed in animals of age P21 and older. P14 animals may possess an intermediate circuitry between P7s with their brief seizure period and the P21-28 animals, whose SE profile is most similar to adult animals. P7 and P14 animals may not be a viable choice for screening anticonvulsant compounds, due to their brief seizure periods.

Age	Dose (mg/kg)	n electrographic seizure	% EEG seizure activity	% mortality (24 hrs)
P7	4	3	33	0
	4.5	3	100	0
P14	4	6	100	33
	4.5	4	100	25
P21	5	12	92	8
	5.5	3	100	0
	6	6	66	33
P28	3	3	0	0
	4	7	57	14
	5.5	4	100	50
	6	8	100	25
	6.5	12	58	42

Table 3. Summary of DFP dose-ranging studies in telemetry-implanted P7-P28 rat pups.

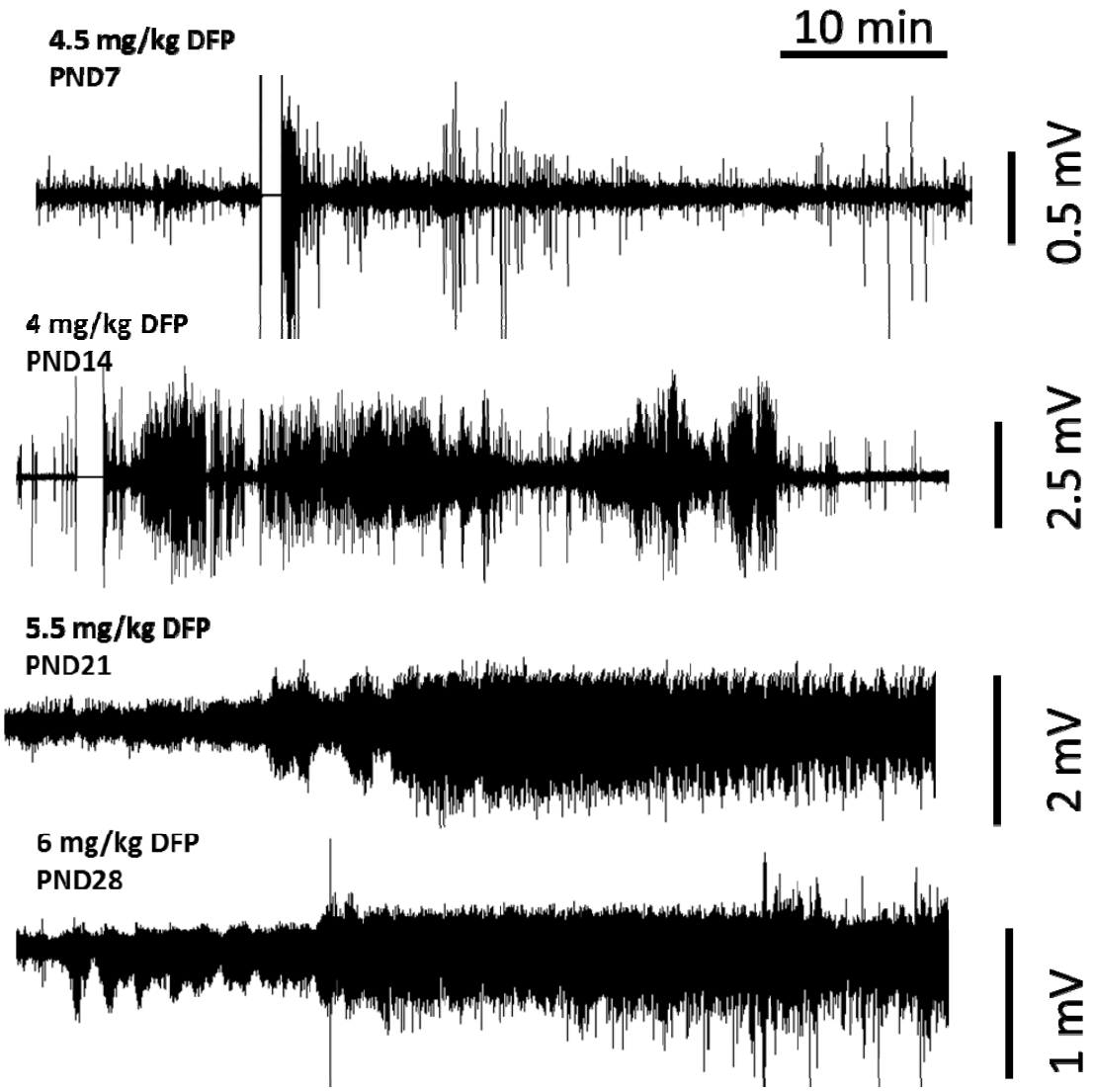

Figure 2. DFP-induced status epilepticus in P7-P28 rat. Animals were treated with 0.026 mg/kg pyridostigmine bromide (i.p.) 30 min prior to dosing with DFP (s.c.). At 1 min following DFP administration, animals were given 0.1 mg/kg atropine sulfate admixed with 25 mg/kg 2-PAM (i.p.).

P28 animals that were treated with DFP and experienced both behavioral and electrographic seizures exhibited extensive signs of neuronal injury (Fig 3). Control animals displayed no FluoroJade B staining (data not shown). In addition to the regions shown in Fig 3, the piriform cortex, endopiriform, and other thalamic nuclei were heavily stained. Lighter staining was observed in the neocortex and hypothalamus.

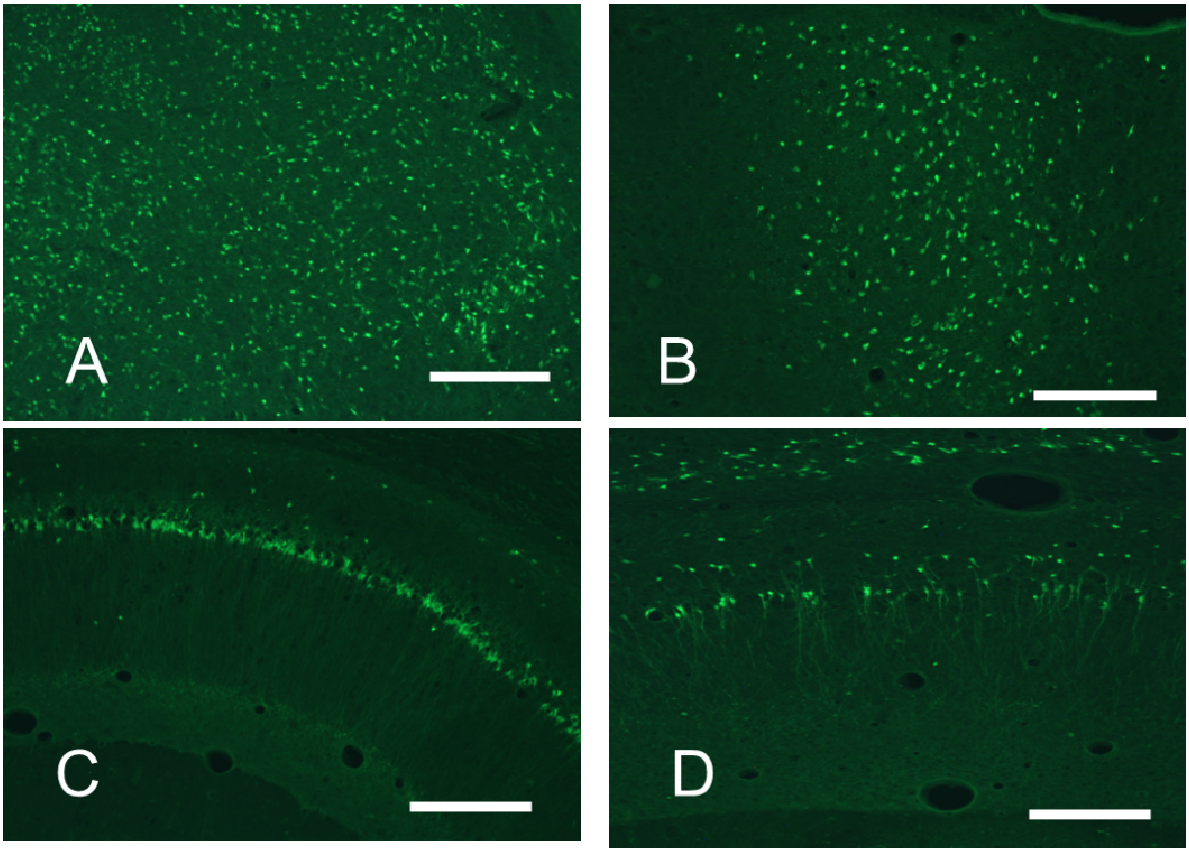

Figure 3. FluoroJade B staining of DFP-treated P28 rats. A. representative image of the dorsomedial amygdala, B. representative image of the mediodorsal thalamic nuclei, C. representative image of the dorsal CA1 region, and D., representative image of the ventral CA1 region. The calibration bar in the bottom right corner corresponds to 100 µm.

A non-linear mixed effects analysis of P28 and adult rats treated with DFP shows a decrease in power in the gamma band in the juvenile animals compared to the adults (Fig. 4). As of this writing, a similar analysis can be performed in P21 animals, and data from P7 and P14 animals is being examined for suitability for fitting.

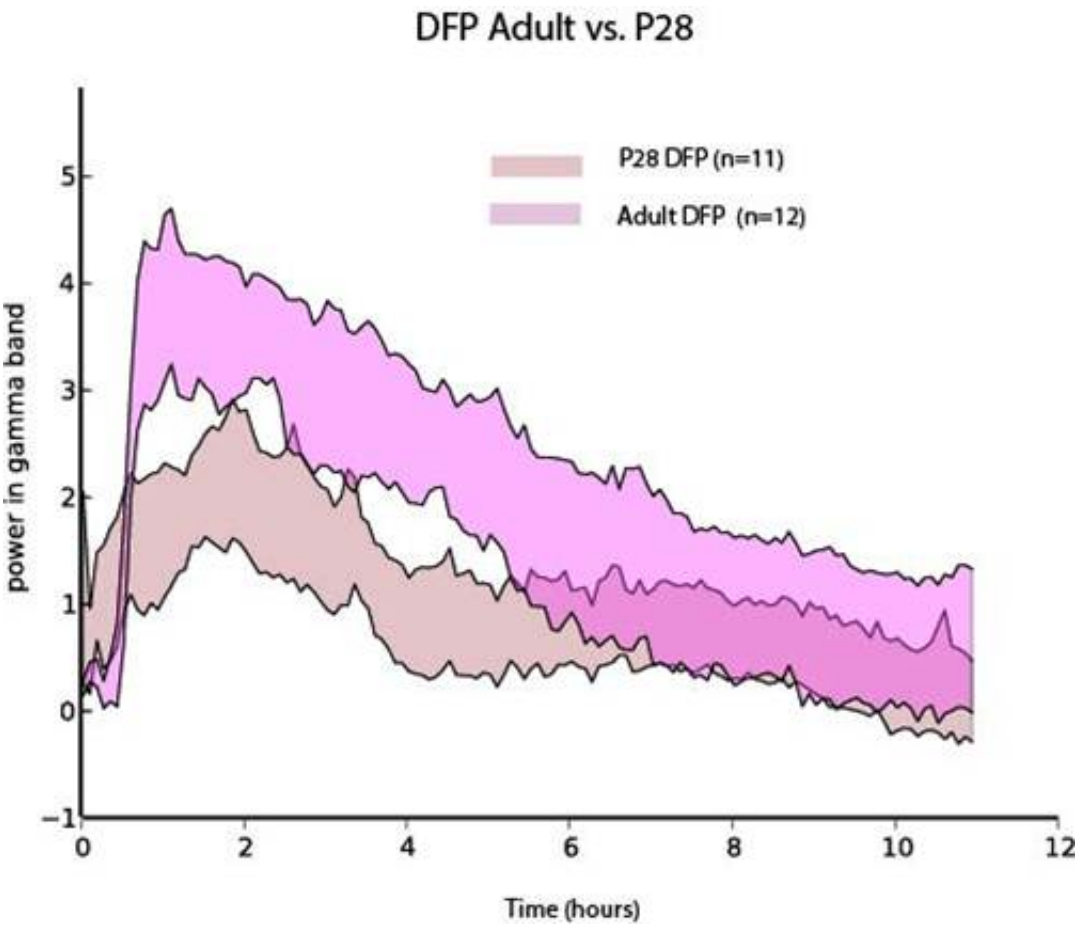

Figure 4. Change in power in the gamma band (20–70 Hz) together with 95% confidence interval over the ten hours following treatment with DFP in both adult and P28 animals.

We have begun analyzing anticonvulsants in P21 and P28 animals. Figure 5 shows an EEG trace of a P21 animal in SE and the anticonvulsant effects of 2 mg/kg midazolam. Midazolam is efficacious in halting both behavioral and electrographic seizures. The EEG from midazolam-treated animals will be analyzed to examine the quality of midazolam's anticonvulsant effects. As only P21 and P28 animals display robust SE, these age groups will be tested with other compounds to assess anticonvulsant efficacy.

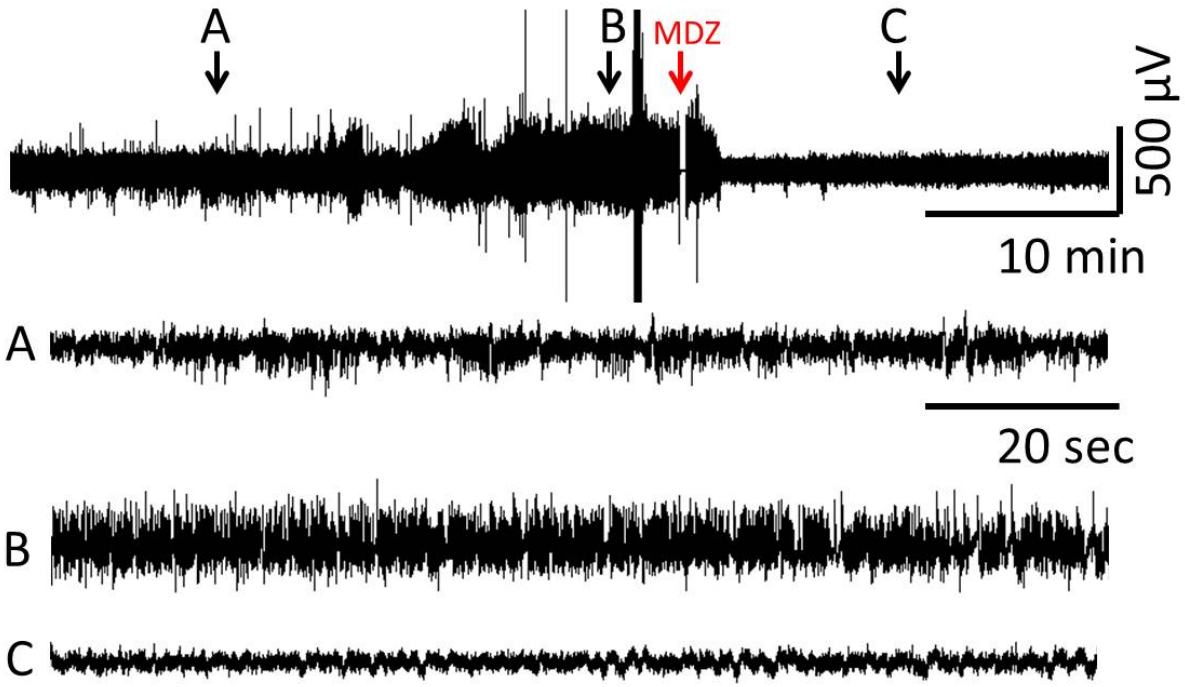

Figure 5. Efficacy of midazolam in ameliorating SE in P21 rats. A and B represent expanded time scale for tracings during SE, C shows the EEG trace after 2 mg/kg midazolam (i.p.).

Key Research Accomplishments

- Provided investigators at the USAMRICD were provided with miniature telemetry devices and recording apparatus
- Established behavior of P7, P14, P21, and P28 animals in response to DFP
- Developed model of organophosphate intoxication (DFP) in telemetry implanted P7, P14, P21, and P28 animals
- Analyzed EEG of DFP-treated immature animals
- Examined neuropathology of DFP-treated immature animals using Fluoro-Jade B
- Began assessment of midazolam in DFP-treated P21 and P28 rats

Reportable Outcomes

Poster: Society of Toxicology meeting in San Antonio, TX, March 10-14, 2013; Scholl, EA, Lehmkuhle, M., McDonough, J., Dudek, FE. A Pediatric Model of Organophosphate-Induced Status Epilepticus in Freely Moving Juvenile Rats.

Poster: CounterACT meeting, Bethesda, MD, June 25-27, 2013; Miller, SM, Scholl, EA, McDonough, J, and Dudek, FE. Development of Rodent Models of Childhood Seizures After Exposure to Organophosphates or Nerve Agents.

Conclusion

Seizure behavior is observed in ages P7-P28 in response to the organophosphate DFP. P7 rat pups sometimes show electrographic seizure activity and P14 pups show a longer electrographic signature, however only P21 and P28 exhibit robust electrographic SE. Initial studies with Fluoro-Jade B show extensive injury in P28 rats, particularly in the hippocampus, thalamus, and amygdala. Pharmacological evaluation of anticonvulsant compounds is being carried out in P21 and P28 rats. Early studies indicate midazolam is efficacious in halting both electrographic and behavioral SE in P21 rats.